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(21) International Application Number: PCT/GB99/02446		(74) Agents: NICHOLLS, Kathryn, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).	
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(71) Applicant (for all designated States except US): ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P ANGELETTI S.P.A. [IT/IT]; Via Pontina Km 30.600, I-00040 Pomezia (IT).			
(71) Applicant (for MN only): NICHOLLS, Kathryn, Margaret [GB/GB]; 32 Somerset Street, Bristol BS2 8LY (GB).			
(72) Inventors; and (75) Inventors/Applicants (for US only): ALTAMURA, Sergio [IT/IT]; Viale Tito Livio, 95, I-00136 Rome (IT). TOMEI, Licia [IT/IT]; Via C.E. Gadda, 173, I-00143 Rome (IT). KOCH, Uwe [DE/IT]; Via Orti Pompei, 23, I-00041 Albano Laziale (IT). NEUNER, Philippe, Jean, Siegfried [FR/IT]; Via Pratolungo, 11B, I-00041 Albano Laziale (IT). SUMMA, Vincenzo [IT/IT]; Via Panoramica, 36, I-00049 Velletri (IT).		Published With international search report.	
(54) Title: DIKETOACID-DERIVATIVES AS INHIBITORS OF POLYMERASES			
<div style="text-align: center;"> <p style="text-align: right;">(A)</p> </div>			
(57) Abstract			
<p>Diketoacids of Formula (A) are useful as inhibitors of viral polymerases. In particular hepatitis C virus RNA dependent RNA polymerase (HCV RdRp), hepatitis B virus polymerase (HBV pol) and reverse transcriptase of human immunodeficiency virus (HIV RT). The group R may be broadly chosen and is an organic moiety which contains 2 to 24 carbon atoms and includes an optionally cyclic or heterocyclic group in which the atom directly bonded to the adjacent carbonyl in the diketoacid is part of the ring structure.</p>			

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5 DIKETOACID-DERIVATIVES AS INHIBITORS OF POLYMERASES

Technical Field

The present invention relates to compounds useful as enzyme inhibitors, in particular as inhibitors of enzymes involved in the transfer of phosphoryl groups and, especially as inhibitors of polymerases. The invention further relates to pharmaceutical compositions containing such compounds, and to their use in the treatment of viral infections.

15

Polymerases are the enzymes which catalyse the formation of phosphodiester bonds in RNA and DNA. They play an essential role in viral replication and, therefore, are an important target in the fight against viral diseases such as human immunodeficiency virus (HIV), hepatitis, and poliomyelitis.

20

Background Art

US 5 475 109 describes dioxobutanoic acids substituted with piperidine or similar N-substituted saturated cycloalkyls as inhibitors of the cap-dependent endonuclease of influenza virus.

25

Disclosure of the Invention

The present inventors have discovered that a range of

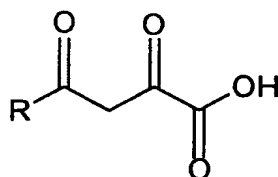
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5 diketoacids have utility as enzyme inhibitors and, in particular, as polymerase inhibitors and more particularly as inhibitors of hepatitis C NS5 RNA-dependent RNA polymerase, HBV DNA-dependent RNA polymerase and HIV DNA- dependent DNA polymerase. Their
10 investigations indicate that these compounds may act by interfering with the binding of phosphoryl groups at the active site of the enzyme and may, therefore, have broad application in inhibiting enzymes involved in the transfer of phosphoryl groups.

15

According to a first aspect of the present invention there is provided a compound of formula A shown below. This compound is suitable for therapeutic use, for instance as an enzyme inhibitor.

20



FORMULA A

Optionally, the compound may be in the form of a pharmaceutically acceptable salt or ester, which can be
25 hydrolysed in vivo to the corresponding diketoacid.

5 In formula A, the group R is an organic moiety which contains from 2 to 24, preferably 4 to 20, most preferably 6 to 17 carbon atoms in total. R includes an optionally substituted cyclic or heterocyclic group in which the atom directly bonded to the adjacent carbonyl
10 in the diketoacid is part of the ring structure. Preferably, this atom is a carbon atom.

The ring which is thus bonded to the carbonyl group is preferably a 3 to 8 membered ring, particularly a 4 to 6
15 membered ring.

Thus, for example, R may be selected from:

(i) optionally substituted aromatic groups,
20 especially those including six membered rings, such as phenyl and naphthyl;

(ii) optionally substituted heteroaryl groups especially those including five and six
25 membered rings such as thiophene, pyrrole, furan, imidazole, pyridyl, pyrimidyl, and pyridazyl; the heteroaryl ring may, optionally be fused to another ring;

30 (iii) optionally substituted cycloalkyl groups,

5 especially those including five or six membered rings such as cyclopentyl, cyclohexyl and adamantyl;

(iv) optionally substituted cycloalkenyl groups, especially those including five or six numbered rings such as cyclohexenyl, cyclopentenyl;

(v) optionally substituted cyclic heteroalkyl groups, especially those including five or six numbered rings such as piperidyl, pyrrolidyl, tetrahydrofuranyl, and tetrahydropyranyl; in this class 4- piperidyl rings substituted with an aryl group at carbon 4 and on acyl or sulfonyl substituent at N1 are preferred.

20

In the case of optional substitution, one or more substituents may be present and a wide variety of substituents are possible. Preferred optional substituents for all compounds of the present invention are set out in the following list:

- (a) -OH;
- (b) -SH;
- (c) - halogen, such as fluorine, chlorine or bromine,
- (d) - CO₂H;

30

- 5 (e) - CN;
- (f) - NO₂ ;
- (g) - NR₁R₂ wherein each of R₁ and R₂ is selected from H and lower alkyl groups having 1 to 6 carbon atoms; or R₁ and R₂ together form a ring including 4 to 6 carbon atoms;
- 10 (h) - SO₂ NR₁R₂ where R₁ and R₂ are as defined above;
- (i) -CONH₂, -NHCO₂H, or -NHCOCOOH;
- (j) an alkyl (or alkenyl or alkynyl group) group having 1 to 12 (2 to 12) carbon atoms, preferably 1 to 7 (2 to 7) carbon atoms optionally substituted by any one or more of the groups (a) - (i) above and/or optionally interrupted by a group selected from -O-, -S-, -NR₃ -,
- 15
- 20
$$\begin{array}{c} \text{O} \\ || \\ -\text{C}- \end{array}, -\text{CO}_2-, -\text{OCO}-, -\text{CONR}_3-, -\text{NR}_3\text{CONR}_3-, -\text{SO}_2-, -\text{NR}_3\text{SO}_2-, \text{ and } -\text{SO}_2\text{NR}_3-;$$
 where each R₃ independently is H or lower alkyl of 1 to 6 carbon atoms;
- (k) an aryl or heteroaryl group having 2 to 10 carbon atoms optionally substituted with any one or more of groups (a) to (j) above;
- 25
- (l) an aralkyl or heteroaralkyl group having 3 to 16 carbon atoms optionally substituted with any one or more of groups (a) - (j) above and/or in which the
- 30

5 alkyl part of the group is optionally interrupted by
a group selected from

10 $-O-$, $-S-$, $-NR_3-$, $-\overset{\overset{O}{\parallel}}{C}-$, $-CO_2-$, $-OCO-$, $-CONR_3-$, $-NR_3CONR_3-$, $-SO_2-$, $-NR_3SO_2-$, and $-SO_2NR_3-$; where
 R_3 is as defined above;

15 (m) $-\overset{\overset{O}{\parallel}}{C}-R_4$ where R_4 is an alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, or heteroaralkyl group as such groups are defined above at (j), (k) and (l);

20 (n) $-\overset{\overset{O}{\parallel}}{C}-O-R_4$ or $-O-\overset{\overset{O}{\parallel}}{C}-R_4$ where R_4 is as defined above;

(o) $-OR_4$ where R_4 is as defined above;

25 (p) $-\overset{\overset{O}{\parallel}}{C}NHR_4$, $-NH-\overset{\overset{O}{\parallel}}{C}-R_4$ or $-NH-\overset{\overset{O}{\parallel}}{C}-NHR_4$ where R_4 is as defined above;

(q) $-SO_2R_4$ where R_4 is as defined above;

(r) $-NHR_4$ or $-N(R_4)_2$ where R_4 is as defined above;

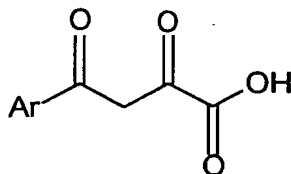
5 (s) $-\text{NHSO}_2\text{R}_4$ or $-\text{SO}_2\text{NHR}_4$, where R_4 is as defined above;

(t) $-\text{SR}_4$

and each of optional substituents (j) to (t) above may
10 optionally itself be substituted by one or more groups
selected from (j) to (t).

A preferred class of compounds of formula A is
represented by formula E:

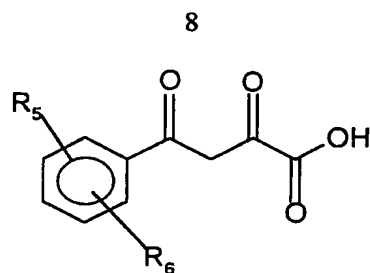
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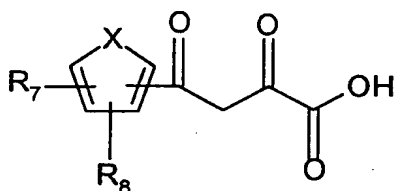
FORMULA E

in which Ar is an optionally substituted aryl or
heteroaryl group. Optional substituents may be selected
20 from the list of preferred substituents set out above.
Within this class of preferred compounds two especially
preferred groups are set out below (formulas F and G)

5



FORMULA F



10

FORMULA G

R_5 , R_6 , R_7 and R_8 are, independently H or are selected from the optional substituents listed above and R_7 and R_8 taken together may form a 4 to 7, preferably 5 or 6 membered ring; and X is O, S, NH, or NR_4 where R_4 is as defined above.

15

In compounds of formula F, (which are optionally substituted phenyl diketoacids) ortho, meta and para

5 substitution are possible.

In general, it is preferred that there is a single substituent, preferably at the position which is ortho- or meta- to the diketoacid group. Substitution at the meta-position is especially preferred. Where two
10 substituents are present, then preferably the phenyldiketoacid is 2,5-substituted; 3,5-substitution is also possible, as is 2,4-substitution provided, in the latter case, that the substituent at the 4-position is
15 relatively small (e.g. methyl). Disubstitution at the 2,3- and 2,6-positions is, in general, not preferred.

Preferred substituents, especially at the ortho and meta positions, are ether groups of formula (o) above (i.e. -OR₄), hydroxyl, and -NHSO₂R₄. It is generally preferred
20 that no more than one substituent be -OR₄ and/or -NHSO₂R₄.

Preferred examples of -OR₄ groups which may be found at the ortho and meta positions and particularly at the meta
25 position include:

-OCH₂Ar or, less preferably -O(CH₂)₂Ar where Ar is an optionally substituted aryl or heteroaryl group and is
30 particularly preferably an optionally substituted phenyl

5 group. Examples of preferred substituents on the aryl group, and especially on the phenyl ring include halogens, especially fluorine and chlorine, and electron-withdrawing groups such as -CN, -CO₂H, and -CF₃, as well as ether and aryl groups;

10 -O-(CH₂)₃-CN; and

-O-(CH₂)₃-C≡CH.

Preferred sulfonamide groups which may be found at the ortho- and meta- positions, particularly at the meta-
15 position are those of formula:

-NH-SO₂-Ar, where Ar is an optionally substituted aryl or heteroaryl group, preferably an optionally substituted phenyl group. Preferred optional substituents for the
20 aryl, preferably phenyl group, include: -CN; halogens, especially chlorine and fluorine, -CF₃, lower (C₁₋₆) alkyl (especially methyl), hydroxy-, ether, and -NO₂ groups.

For both the -OCH₂Ar and -NHSO₂Ar substituted compounds,
25 another preferred example of Ar is naphthyl.

Other preferred substituents at the ortho and meta positions are lower (eg C₁₋₆) alkyl groups, especially C₁₋₄ alkyl, such as methyl and ethyl, but in particular
30 methyl, aralkyl groups, especially phenylmethyl groups,

5 optionally substituted in the phenyl ring, especially by
a halogen, and nitrogen-containing substituents such as
primary, secondary or tertiary amine groups, optionally
in protonated form, amide, urethane, or urea groups in
each of which examples there is a nitrogen atom bonded to
10 the phenyl ring.

One particularly preferred sub class of compounds of
formula F is those in which each of R_5 and R_6 is
selected from H, HO-, R_4 O-, and $-NHSO_2R_4$ provided that
15 no more than one of R_5 and R_6 is R_4 O- or $-NHSO_2R_4$.

In compounds of formula G the diketoacid group may be at
the 2- or 3- position of the ring. In many cases
20 substitution at the 2-position is preferred.

Preferred examples of compounds of formula G are those in
which the five membered aromatic ring,



25 is a pyrrole or thiophene ring. In the case of the
pyrrole-substituted diketoacids, the groups R_7 and R_8 may
both be hydrogen and in many cases that is preferred. If
 R_7 and R_8 correspond to substituent groups, then these may

5 be at any of the positions not already occupied by the
diketoacid group. Examples of possible substituents
include alkyl (especially methyl), halogen, and aralkyl
(especially benzyl) groups.

10 One embodiment of pyrrole substituted diketoacid is that
in which the diketoacid group is at the 2- position of
the ring and where the only other substituent in the ring
is on the nitrogen atom. In this case, preferred
examples of the substituent R_4 present on the nitrogen
15 atom, include alkyl, aryl or aralkyl groups, particularly
aralkyl (such as benzyl) groups. Where an aryl or
aralkyl group is present these are preferably substituted
with halogen atoms, such as fluorine or chlorine, or by
cyano-groups.

20

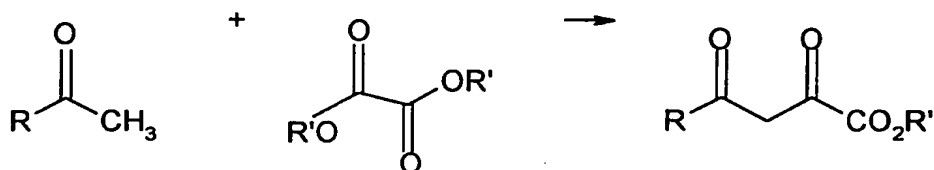
In the case of the thiophene-substituted diketoacids a
wide range of substituents R_7 and R_8 may be employed in
various positions as will be evident from the tables
infra. Preferred thiophenes have an aralkyl (such as
25 optionally substituted benzyl) or aryl (such as
optionally substituted phenyl) substituent, e.g. at the
5-position of the thiophene ring.

Compounds containing furanyl rings may also be useful,
30 especially for inhibiting HIV reverse transcriptase.

5 Preferred substituents are optionally substituted aryl groups (especially optionally substituted phenyl). Substitution is preferably at the 5-position of the ring.

The formulae of numerous preferred specific compounds of
10 the present invention are presented later below.

The compounds of the present invention having formula A may be prepared by a process which comprises reaction of a compound of formula B with a dialkyloxalate of formula C followed by hydrolysis of the resulting diketo-ester of
15 formula D:



FORMULA B

FORMULA C

FORMULA D

20 where R' is an alkyl group, typically having 1-6 carbon atoms. In the case where the target molecule is a pharmaceutically acceptable ester of the compound of formula A then R' in formula C may be selected accordingly, and the step of hydrolysing the compound of
25 formula D omitted, since in vivo hydrolysis can render

5 the compounds active.

Preferred enzymes for inhibition by the compounds of the invention are those involved in phosphate transfer, in particular polymerases such as DNA polymerases, and RNA
10 polymerases both of which may be either RNA dependent or DNA dependant. Compounds of the invention may particularly preferably be employed in the inhibition of viral enzymes. Examples of viral enzymes include RNA - dependent RNA polymerase and reverse transcriptases.

15

The compounds of the invention may be used as inhibitors of plant or animal (including human) viruses.

The viruses may be RNA viruses, which may, for example,
20 be positive single stranded viruses of which polio virus, hepatitis C virus and encephalomyocarditis are examples, negative single stranded viruses such as orthomyxoviruses, and paramyxoviruses, and retroviruses of which HIV is a prominent example. Alternatively, the
25 viruses may be DNA viruses, especially double stranded DNA viruses such as hepatitis B virus. In particular, compounds of the present invention may inhibit one or more of the following enzymes: hepatitis C virus RNA dependent RNA polymerase (HCV RdRp), hepatitis B virus
30 polymerase (HBV pol) and reverse transcriptase of human

5 immunodeficiency virus (HIV RT).

Especially preferred compounds of the invention will be suitable for use as HCV RdRp inhibitors.

10 Other classes of enzyme involved in phosphate transfer which may be susceptible to inhibition by compounds of the present invention include phosphatases, Rnases, integrases and ribozymes.

15 According to a further aspect of the invention there is provided the non-therapeutic use of compound of formula A or suitable salt or ester as an enzyme inhibitor, especially as an inhibitor of polymerases, especially viral polymerases. For instance, compounds of the
20 invention may be of utility in agriculture and horticulture for treating plants infected with or susceptible to plant virus.

According to a further aspect of the invention there is
25 provided the use of a compound of formula A or of a pharmaceutically acceptable salt or ester thereof in the manufacture of a medicament for treatment of a viral illness in a human or animal. For instance, the medicament may be used to treat viral illness by
30 inhibiting one or more viral polymerase. Preferably the

5 medicament is for treatment of hepatitis, such as hepatitis B or C, particularly hepatitis C, and human immunodeficiency virus.

A still further aspect of the invention provides a
10 pharmaceutical composition comprising a compound of formula A, or a pharmaceutically acceptable salt or ester thereof and a pharmaceutically acceptable excipient, diluent or carrier. The composition may be in any suitable form, depending on the intended method of
15 administration. It may for example be in the form of a tablet, capsule or liquid for oral administration, or of a solution or suspension for administration parenterally.

The pharmaceutical compositions optionally also include
20 one or more other agents for the treatment of viral infections such as an antiviral agent, or an immunomodulatory agent such as α -, β -, or γ - interferon.

A still further aspect of the invention provides a method
25 of inhibiting an enzyme, especially a viral polymerase and/or of treating or preventing a viral illness, the method involving administering to a human or animal (preferably mammalian) subject suffering from the condition a therapeutically or prophylactically effective
30 amount of the pharmaceutical composition described above

5 or of a compound of formula A or salt or ester thereof.
"Effective amount" means an amount sufficient to cause a benefit to the subject or at least to cause a change in the subject's condition.

10 The dosage rate at which the compound, salt or ester is administered will depend on the nature of the subject, the nature and severity of the condition, the administration method used, etc. Appropriate values are selectable by routine testing. The compound, salt or
15 ester may be administered alone or in combination with other treatments, either simultaneously or sequentially. For instance, it may be administered in combination with effective amounts of antiviral agents, immunomodulators, anti-infectives, or vaccines known to those of ordinary
20 skill in the art. It may be administered by any suitable route, including orally, intravenously, cutaneously, subcutaneously, etc. It may be administered directly to a suitable site or in a manner in which it targets a particular site, such as a certain type of cell.
25 Suitable targeting methods are already known.

A further aspect of the invention provides a method of preparation of a pharmaceutical composition, involving admixing one or more compound of formula A or salt or
30 ester thereof with one or more pharmaceutically

- 5 acceptable adjuvants, diluents or carriers and/or with one or more other therapeutically or prophylactically active agents.

Modes for Carrying Out the Invention

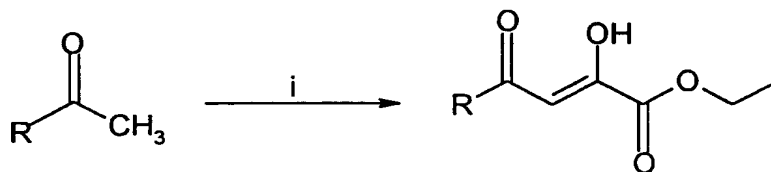
- 10 Embodiments of the invention are described below by the way of example only.

EXAMPLES

- 15 (1) Synthesis

The synthesis of the 2,4-dioxobutanoic acids consists of a Claisen condensation reaction between a methyl ketone substrate and diethyl oxalate in the presence of sodium ethoxide in tetrahydrofuran (Scheme 1A) and the subsequent hydrolysis of the ethyl ester with sodium hydroxide in methanol (Scheme 1B)

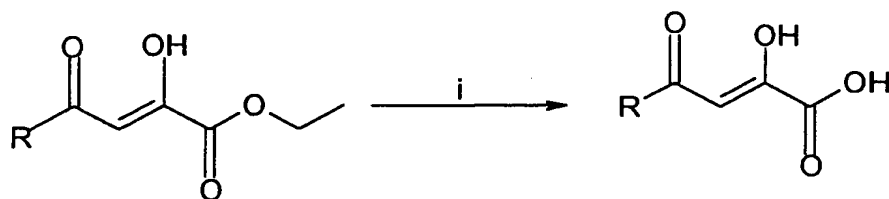
Scheme 1A



Reagents: (i) diethyl oxalate/NaOEt in THF

- 25 Scheme 1B

5



Reagents: (i) 5eq. NaOH/MeOH

Exemplary procedure for the synthesis of the 2, 4 -dioxobutanoate ethyl esters

10 (Scheme 1A)

In a 50 ml round bottom flask with a stirring bar and under an inert atmosphere, the methyl ketone compound (1.0 mmole) in 10 ml of dry tetrahydrofuran (THF) is reacted with 2 equivalents of diethyl oxalate and 2 equivalents of sodium ethoxide (NaOEt) at ambient temperature for 3 hours. When reaction is completed, the reaction mixture is poured into a 1N aqueous hydrochloric acid (HCl) and extracted with ethyl acetate (EtOAc). The organic phase is separated, washed first with water and then with brine. The organic layer is dried over sodium sulfate (Na₂SO₄), filtered and solvent is removed in vacuo leaving the desired dioxobutanoate ethyl ester in quantitative yield.

20

Exemplary procedure for hydrolysis of the ethyl ester

5 (Scheme 1B)

In a 50 ml round bottom flask with a stirring bar, the 2,4-dioxobutanoate ethyl ester compound (1.0 mmole) in 10 ml of methanol (MeOH) is reacted with 5 equivalents of sodium hydroxide (NaOH) at ambient temperature for 2
10 hours.

The methanol is removed in vacuo. The aqueous residue is washed with diethyl ether (Et₂O). The aqueous fraction is acidified by addition of 1N aqueous hydrochloric acid
15 solution (HCl) and the milky mixture is extracted with two portions of ethyl acetate (EtOAc). The combined organic fractions are washed with brine. The organic layer is dried over sodium sulfate (Na₂SO₄), filtered and solvent is removed in vacuo leaving the desired
20 dioxobutanoic acid product.

Using this or analogous methods, compounds were produced as set out in the following Tables, which are categorised according to their "R" group.

25

The Tables include IC₅₀ data and the methods for assay are explained after the Tables.

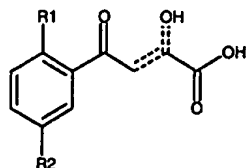
Notes to Table: NA = not active as an inhibitor at
30 concentrations up to that stated.

ND = not done.

In the tables, where nitrogen atoms appear to be divalent, the presence of a hydrogen atom is implied.

Tabl I

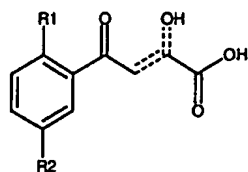
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
1	X_1-H	X_2-H	5.6
2	X_1-CH_3	X_2-H	3
3	X_1-H		27.9
4	X_1-H		8
5	X_1-H		17
6		X_2-H	18
7	X_1-H		2.92
8		X_2-H	44

Table I

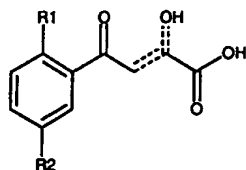
HCV-polymerase inhibitors: examples of 2,5-substituted
phenylidiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
9			51
10			20
11			7.08
12			16.7
13			2.6
14			26
15			83.5

Table I

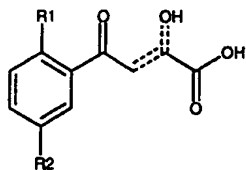
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
16			4.3
17			11.6
18			2.2
19			11.9
20			0.38
21			0.955

Table I

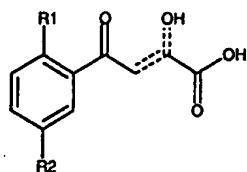
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
22	X_1-H		19
23	X_1-H	$HO-X_2$	0.94
24	X_1-H		19
25		X_2-H	28
26		X_2-H	26

Table I

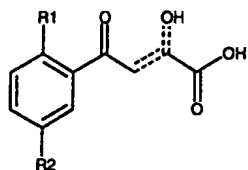
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
27		X_2-H	2.84
28	X_1-H		6.2
29		X_2-H	3.9
30	X_1-H		15
31		X_2-H	18

Table I

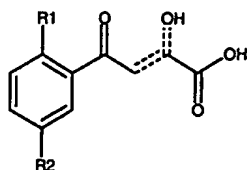
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
32			6.1
33			18.2
34			9.6
35			6.1
36			1.6
37			18

Table I

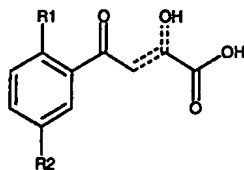
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
38		X_2-H	16
39		X_2-H	22
40		X_2-H	8.3
41	X_1-H		28.9
42		X_2-H	16.6
43	X_1-H		20
44	X_1-H		18.5

Table I

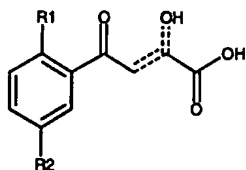
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
45	X_1-H		12.9
46	X_1-H		30.1
47	X_1-H		20.7
48	X_1-H		22
49	X_1-H		32
50		X_2-H	7.8

Table I

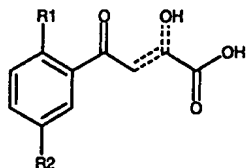
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
51		X_2-H	1.9
52		X_2-H	10
53		X_2-OH	0.115
54		X_2-Br	2.3
55		X_2-H	10.8

Table I

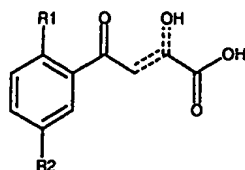
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
56			23.6
57			2.1
58			13.6
59		X_2-H	25.3
60	X_1-H	H_3C-O-X_2	40

Table I

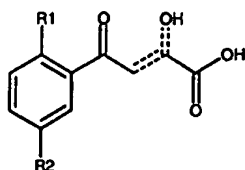
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
61	X_1-H		31
62	X_1-H	H_2N-X_2	10
63		$H_3N^+-X_2$	1.7
64	X_1-H		0.23
65		X_2-H	45
66	X_1-H		11

Table I

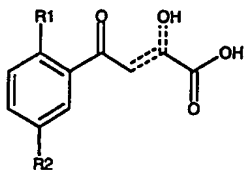
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
67	X_1-H		16
68	X_1-H		30
69		X_2-H	14
70	X_1-H		9.2

Table I

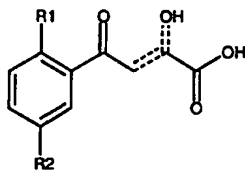
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiket acids



Ex. No.	R1	R2	IC 50 (μM)
71	X_1-H		10.6
72	X_1-H		0.48
73	X_1-H		5.6
74	X_1-H		3.6
75	X_1-H		19.2
76	X_1-H		50

Table I

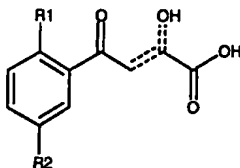
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
77	X ₁ -H		4.8
78	X ₁ -H		0.67
79	X ₁ -H		6
80	X ₁ -H		3
81	X ₁ -H		1.4

Table I

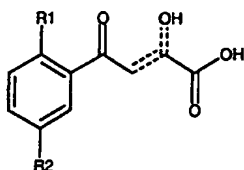
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
82	X ₁ -H		19
83	X ₁ -H		9.4
84	X ₁ -H		0.95
85	X ₁ -H		13
86	X ₁ -H		2.05

Table I

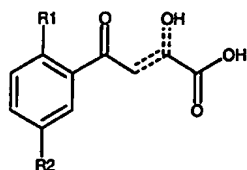
HCV-polymerase inhibitors: examples of 2,5-substituted
ph nyldiket acids



Ex. No.	R1	R2	IC 50 (μM)
87	X_1-H		2.3
88	X_1-H		0.7
89	X_1-H		3.3
90	X_1-H		1.8
91	X_1-H		6.2

Tabl I

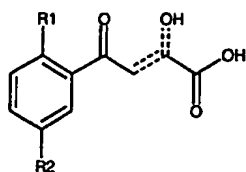
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
92	X_1-H	 <chem>COc1ccccc1COX2</chem>	1
93	X_1-H	 <chem>Fc1ccccc1C(F)(F)FCOX2</chem>	1.9
94	X_1-H	 <chem>Fc1ccccc1COX2</chem>	5.8
95	X_1-H	 <chem>Clc1cc(s1)C(Cl)=C1NS(=O)(=O)X2</chem>	0.48

Table I

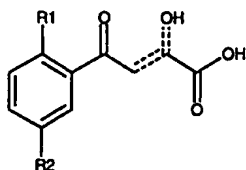
HCV-polymerase inhibitors: examples of 2,5-substituted
ph nylidic toacids



Ex. No.	R1	R2	IC 50 (μM)
96	X_1-H		50
97	X_1-H		2.8
98	X_1-H		1
99	X_1-H		0.6

Table I

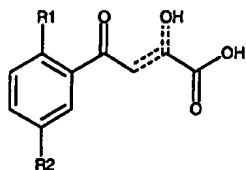
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
100	X_1-H		7.8
101	X_1-H		7
102	X_1-H		1.5
103	X_1-H		6
104	X_1-H		50

Table I

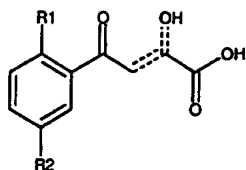
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
105	X_1-H		13.7
106	X_1-H		6.8
107	X_1-H		0.14
108	X_1-H		6.9
109	X_1-H		0.17

Table I

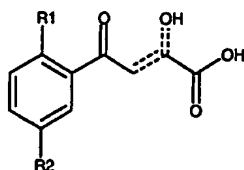
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
110	$X_1 - H$		30
111	$X_1 - H$		0.12
112	$X_1 - H$		1.33
113	$X_1 - H$		0.1
114	$X_1 - H$		0.5

Table I

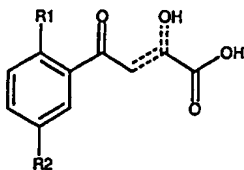
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
115	X ₁ -H		3.7
116	X ₁ -H		0.3
117	X ₁ -H		0.14
118	X ₁ -H		0.2
119	X ₁ -H		0.049

Tabl I

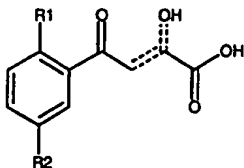
HCV-polym rase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
120	X_1-H		0.36
121	X_1-H		4
122	X_1-H		2
123	X_1-H		0.29

Tabl I

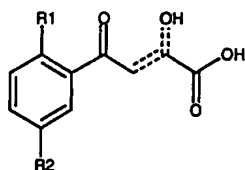
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
124	X_1-H		28
125	X_1-H		0.17
126	X_1-H		0.056
127	X_1-H		0.3

Tabl I

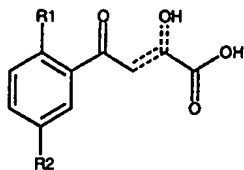
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
128	$X_1 - H$		24
129	$X_1 - H$		1.6
130	$X_1 - H$		0.14
131	$X_1 - H$		0.78
132	$X_1 - H$		0.67

Tabl I

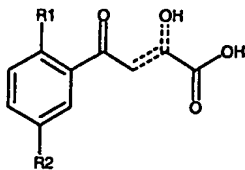
HCV-polymeras inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
133	X_1-H		3.2
134	X_1-H		23
135	X_1-H		21
136	X_1-H		0.2

Table I

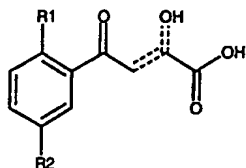
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
137	X ₁ -H		0.9
138	X ₁ -H		1.1
139	X ₁ -H		1.4
140	X ₁ -H		1
141	X ₁ -H		0.56

Tabl I

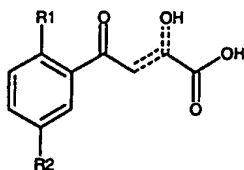
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
142	X ₁ -H		0.4
143	X ₁ -H		0.45
144	X ₁ -H		14
145	X ₁ -H		1.2
146	X ₁ -H		15

Table I

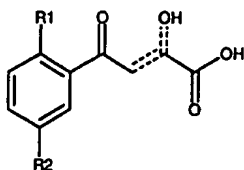
HCV-polym rase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
147	X_1-H		1.3
148	X_1-H		0.26
149	X_1-H		0.55
150	X_1-H		2.3

Table I

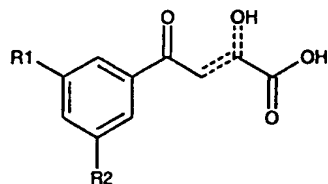
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
151	X_1-H	<p>X_2 N-SO₂- Cl Cl</p>	0.5
152	X_1-F	<p>X_2 -CH₂- C₆H₅</p>	20
153	X_1-H	<p>X_2 N=N N-CH₂- C₆H₄</p>	19
154	X_1-H	<p>X_2 N=N=N N-CH₂- C₆H₄</p>	30

Tabl II

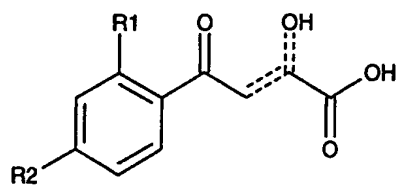
HCV-polymerase inhibitors: examples of 3,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
155			1.4
156			1.3
157			0.9
158			0.2
159			20
160			0.1

TABLE III

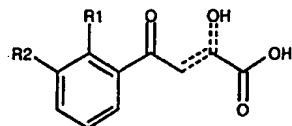
HCV-polymerase inhibitors: examples of 2,4-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
161	H-X ₁	H ₃ C-X ₂	2.8
162	H-X ₁	HO-X ₂	5.5
163	H-X ₁	F-X ₂	26
164	H-X ₁	H ₃ C-CH ₂ -X ₂	47
165	$\begin{array}{c} \text{CH}_3 \\ \\ \text{X}_1 \end{array}$	H ₃ C-X ₂	2
166	H-X ₁	Cl-X ₂	20
167	$\begin{array}{c} \text{N} \\ \\ \text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2- \\ \\ \text{X}_1 \end{array}$	H ₃ C-X ₂	0.6

Table IV

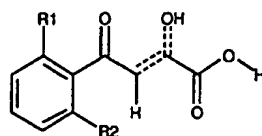
HCV-polymerase inhibitors: examples of 2,3-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
168			18
169			>50
170			>50

Tabl V

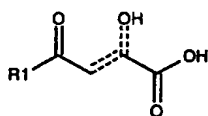
HCV-polymeras inhibitors: examples of 2,6-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
171			12
172			>50

Table VIa

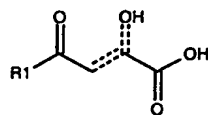
HCV-polymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
173		21
174		13.4
175		25
176		29
177		25

Table VIa

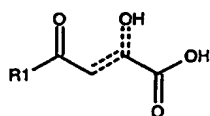
HCV-polymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
178		17.9
179		12.8
180		93
181		30
182		30

Table VIa

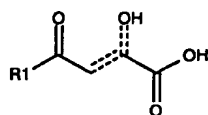
HCV-p lymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
183	 <chem>X1-c1cc[nH]c1Cc2ccc(C#N)cc2</chem>	32
184	 <chem>X1-c1cc[nH]c1Cc2ccc(C#N)cc2</chem>	6.7
185	 <chem>X1-c1cc[nH]c1Cc2ccccc2C#N</chem>	6.3
186	 <chem>X1-c1cc[nH]c1Cc2ccccc2Cl</chem>	24

Table VIa

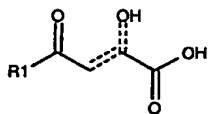
HCV-polymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
187	<p>Chemical structure of R1 for example 187: A pyrrole ring with a substituent X₁ at the 3-position, connected via a methylene group to a 4-fluorophenyl ring.</p>	36
188	<p>Chemical structure of R1 for example 188: A naphthalene ring system connected via a methylene group to a pyrrole ring with a substituent X₁ at the 3-position.</p>	12.7
189	<p>Chemical structure of R1 for example 189: A phenyl ring connected via an ether linkage to a 4-pyrrolylmethyl group, which is further connected to a pyrrole ring with a substituent X₁ at the 3-position.</p>	28
190	<p>Chemical structure of R1 for example 190: A 4-fluorophenyl ring connected via a methylene group to a pyrrole ring with a substituent X₁ at the 3-position and an iodine atom at the 4-position.</p>	18

Table VIb

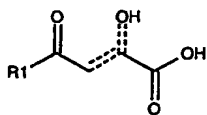
HCV-polymerase inhibitors: xamples of thiophen -2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
191		10
192		8.2
193		12
194		16
195		11.1
196		15
197		11
198		7.9

Table VIb

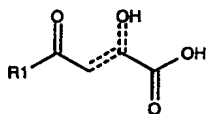
HCV-polymerase inhibitors: examples of thiophene-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
199		17
200		8.2
201		20
202		68
203		19.8
204		11
205		74
206		65

Tabl Vib

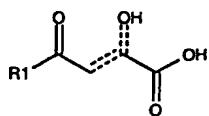
HCV-polymerase inhibitors: examples of thiophene-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
207	 <chem>Fc1ccc(cc1)Cc2cc(s2)X1</chem>	9.9
208	 <chem>Fc1ccc(cc1)Cc2cc(s2)X1</chem>	11.6
209	 <chem>c1ccccc1S2C=CC(S2)X1</chem>	12.6
210	 <chem>Fc1ccc2c(c1)oc3cc(s3)X1</chem>	27
211	 <chem>Clc1ccc(cc1)Cc2cc(s2)X1</chem>	82

Table VIb

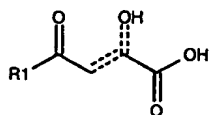
HCV-polymerase inhibitors: examples of thiophene-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
212		7.5
213		5.9
214		17
215		15.3

Table VIc

HCV-polymerase inhibitors: examples of furan-2-substituted diketoacids



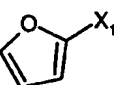
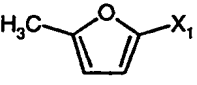
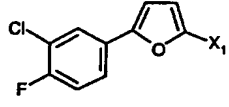
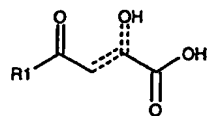
Ex.	R1	IC 50 (μM)
216		50
217		58
218		41.2

Table VIIa

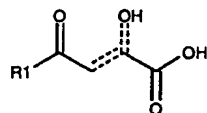
HCV-polymerase inhibitors: examples of pyrrole-3-substituted diketoacids



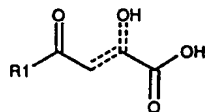
Ex.No.	R1	IC 50 (μM)
219		23.7
220		4.6
221		20.6

Table VIIb

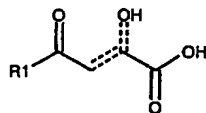
HCV-p lymerase inhibitors: examples of
thiophene-3-substituted diketoacids



Ex.No.	R1	IC 50 (μM)
222		4
223		27
224		50
225		167
226		17
227		15
228		17.8

Table VIIbHCV-polymerase inhibitors: examples of
thiophene-3-substituted diketoacids

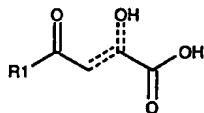
Ex.No.	R1	IC 50 (μM)
229		80
230		8.6
231		9.4
232		11.8
233		9.2
234		14.5

Table VIIfbHCV-polymerase inhibitors: xamples of
thiophene-3-substituted diketoacids

Ex.No.	R1	IC 50 (μM)
235	<p>Chemical structure of R1 for example 235: A thiophene ring substituted at the 3-position with a 4-chlorophenylthio group (-S-C₆H₄-Cl) and at the 5-position with an X₁ group.</p>	7.5
236	<p>Chemical structure of R1 for example 236: A benzothiophene ring system substituted at the 3-position with an X₁ group.</p>	26

Table VIIC

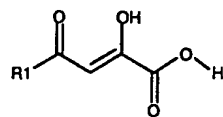
HCV-polymerase inhibitors: examples of furan-3-substituted diketoacids



Ex.No.	R1	IC 50 (μM)
237	<p>Chemical structure of R1 for example 237: A furan ring with a methyl group (CH_3) at position 2, a substituent X_1 at position 3, and a methyl group (H_3C) at position 4.</p>	14
238	<p>Chemical structure of R1 for example 238: A furan ring with a methyl group (CH_3) at position 2, a substituent X_1 at position 3, and a methyl group (CH_3) at position 4.</p>	47.5

Table VIII

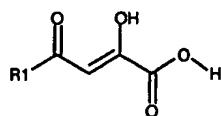
HCV-polymerase inhibitors: examples of alkyl-diketoacids



Ex. No.	R1	IC 50 (μM)
239		9.4
240		18
241		37
242		12.8
243		6.7
244		77
245		81.4

Table VIII

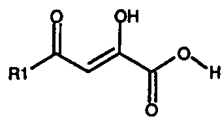
HCV-polymerase inhibitors: xamples of alkyl-diketoacids



Ex. No.	R1	IC 50 (μM)
246		18
247		45
248		10
249		60
250		17
251		21

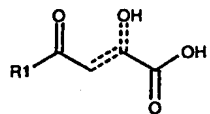
Table VIII

HCV-polymerase inhibitors: examples of alkyl-diketoacids



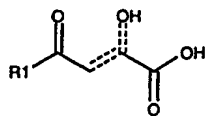
Ex. No.	R1	IC 50 (μM)
252		61
253		55
254		14
255		16.7
256		25
257		50

Table IXa
most active HCV-inhibitors



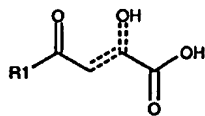
Ex. No.	R1	HCV	HIV	HBV
126	 <chem>X1c1ccc(OCC2=CC=C(C#N)C(Cl)=C2)cc1</chem>	0.056	100	ND
160	 <chem>Oc1ccc(X1)cc1OCC2=CC=C(C#N)C(Cl)=C2</chem>	0.1	NA	ND
113	 <chem>X1c1ccc(OCC2=CC=C(C#N)C(Br)=C2)cc1</chem>	0.1	90	ND
53	 <chem>N#CCCCOc1ccc(X1)cc1O</chem>	0.115	37	ND

Table IXa
most active HCV-inhibitors



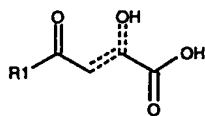
Ex. No.	R1	HCV	HIV	HBV
111		0.12	80	ND
107		0.14	58	ND
117		0.14	100	ND
109		0.17	NA	ND

Table IXa
most active HCV-inhibitors



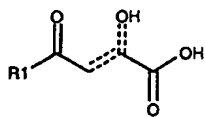
Ex. No.	R1	HCV	HIV	HBV
158		0.2	NA	ND
64		0.23	NA	ND
116		0.3	NA	ND
120		0.36	80	ND

Table IXa
most active HCV-inhibitors



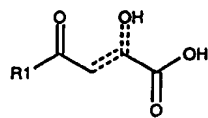
Ex. No.	R1	HCV	HIV	HBV
20		0.38	27	ND
72		0.48	NA	ND
99		0.6	50	ND
78		0.67	35	ND

Table IXa
most active HCV-inhibitors



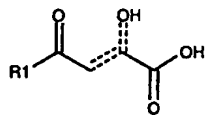
Ex. No.	R1	HCV	HIV	HBV
88		0.7	NA	ND
84		0.95	NA	ND
21		1	>50	ND
23		1	59	ND

Table IXa
most active HCV-inhibitors



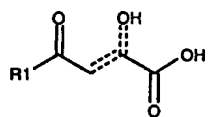
Ex. No.	R1	HCV	HIV	HBV
112		1.33	90	ND
155		1.4	130	416
36		1.6	24	ND
90		1.8	NA	ND
165		2	NA	ND

Table IXa
most active HCV-inhibitors



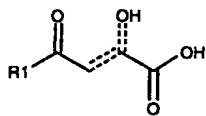
Ex. No.	R1	HCV	HIV	HBV
18		2.2	30	ND
161		2.8	320	108
80		3	NA	ND
27		3	>50	ND
7		3.3	61	6

Table IXa
most active HCV-inhibitors



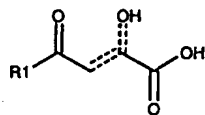
Ex. No.	R1	HCV	HIV	HBV
16		4.3	>100	ND
162		5.5	NA	ND
1		5.6	90	NA
103		6	NA	ND
243		6.7	26.8	ND

Table IXa
most active HCV-inhibitors



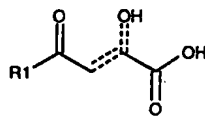
Ex. No.	R1	HCV	HIV	HBV
198		7.9	NA	ND
4		8	>100	ND
192		8.2	NA	ND
66		11	NA	ND
19		12	77	ND
179		12.8	NA	NA

Tabl IXa
most active HCV-inhibitors



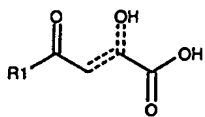
Ex. No.	R1	HCV	HIV	HBV
190	<p>The structure shows a 4-fluorophenyl group attached to the nitrogen of a 2-iodo-5-substituted pyrrole ring. The substituent at the 5-position is X₁.</p>	18	NA	NA
24	<p>The structure shows a benzamide derivative where the benzene ring is substituted with X₁ at the para position relative to the amide group.</p>	19	71	ND
49	<p>The structure shows a biphenyl system where one phenyl ring is substituted with two bromine atoms (Br) at the 3 and 4 positions, and the other phenyl ring is substituted with X₁ at the para position.</p>	32	NA	ND

Table IXb
most active HBV-Pol-inhibitors



Ex. No.	R1	HCV	HIV	HBV
206		65	NA	2
205		74	NA	3.3
225		167	86	4
202		70	>100	9
196		15	50	9

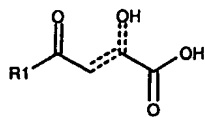
Table IXc
most active HIV-RT-inhibitors



Ex. No.	R1	HCV	HIV	HBV
258		>100	3.6	NA
218		41.2	11.8	40
259		>100	16	NA
40		8.3	12	NA
20		0.38	27	ND

Table IXc

most active HIV-RT-inhibitors



Ex. No.	R1	HCV	HIV	HBV
8	<p>Chemical structure of the R1 substituent for Example 8, showing a benzyl group linked via an oxygen atom to a phenyl ring with a substituent X1.</p>	44	19	ND

5 2. Measurement of Inhibitory Activity

The effectiveness of the compounds set out above as polymerase inhibitors, stated above as IC₅₀ values, was assessed in screening assays as follows.

10 In initial tests, the compounds were tested to see if they were effective as inhibitors of the RNA-dependent RNA polymerase (RdRp) of hepatitis C virus (HCV). The HCV NS5B protein is the viral RdRp; compounds capable of interfering with the activity of this enzyme are thus
15 expected to block viral replication.

Test for Inhibition of Hepatitis C Virus RdRp

WO96/37619 describes the production of recombinant HCV
20 RdRp from insect cells infected with recombinant baculovirus encoding the enzyme. The purified enzyme was shown to possess in vitro RNA polymerase activity using RNA as template. The reference describes a
25 polymerisation assay using poly (A) as a template and oligo(U) as a primer. Incorporation of tritiated UTP is quantified by measuring acid-insoluble radioactivity. The present inventors have employed this assay to screen the various compounds described above as inhibitors of HCV RdRp and other virally encoded polymerases.

5 Incorporation of radioactive UMP was measured as follows.
The standard reaction (100 μ l) was carried out in a
buffer containing 20mM tris/HCl pH 7.5, 5mM MgCl₂, 1mM
DTT, 50mM NaCl, 1mM EDTA, 20U Rnasin (Promega), 0.05%
Triton X-100, 1 μ Ci[³H] UTP (40 Ci/mmol, NEN), 10 μ M UTP and
10 10 μ g/ml poly(A). Oligo (U)₁₂ (1 μ g/ml, Genset) was added
as a primer. The final NSSB enzyme concentration was
20 nM. After 1 hour incubation at 22 °C the reaction was
stopped by adding 100 μ l of 20% TCA and applying samples
to DE81 filters. The filters were washed thoroughly with
15 5% TCA containing 1M Na₂HPO₄/NaH₂PO₄, pH 7.0, rinsed
with water and then ethanol, air dried, and the filter-
bound radioactivity was measured in the scintillation
counter. By carrying out the reaction in the presence of
various concentrations of each of the compounds set out
20 above it was possible to determine IC₅₀ values for each
compound with the formula:

$$\% \text{ residual activity} = 100 / (1 + [I] / IC_{50})^s$$

where [I] is the inhibitor concentration and "s" is the
slope of the inhibition curve.

25

Test for Inhibition of Hepatitis B Virus Polymerase

Analogous assays employed the polymerase of hepatitis B
virus (HBV pol), obtained in the form of viral particles
30 from the sera of HBV positive patients. These particles

5 contain a polymerase bound to an incomplete double stranded DNA template. In the assay the incorporation of ^{32}P -dNTP is measured as radioactivity incorporated in acid insoluble precipitate.

The standard reaction (100 μl) was carried out in a
10 buffer containing 50mM tris/HCl pH 7.5, 30mM MgCl₂, 1mM DTT, 100 mM KCl, 0.02% Triton X-100, 1 μCi [^{32}P] dCTP (300 Ci/mmol, NEN), 1 μM dATP, dTTP, dGTP. After 1 hour incubation at 37 °C the reaction was stopped by adding 100 μl of 20% TCA and applying samples to DE81 filters. The
15 filters were processed and IC₅₀ values calculated as described above.

Test for Inhibition of Human Immunodeficiency Virus-1
Reverse Transcriptase

20

Analogous assays employed the reverse transcriptase of HIV (HIV -1RT) from Boehringer Mannheim.

Incorporation of radioactive dTTP was measured as
25 follows. The standard reaction (100 μl) was carried out in a buffer containing 50mM tris/HCl pH 8.2, 2.5mM MgCl₂, 1mM DTT, 80 mM KCl, 5mM EGTA, 0.05% Triton X-100, 1 μCi [^3H] dTTP (40 Ci/mmol, NEN), 10 μM UTP and 10 $\mu\text{g/ml}$ poly(A)/dT (from Pharmacia). The final HIV-1RT(enzyme
30 concentration was 1 nM. After 1 hour incubation at 37 °C

5 the reaction was stopped by adding 100 μ l of 20% TCA and
applying samples to DE81 filters. The filters were
processed and IC₅₀ values calculated as described above.

The results demonstrate that the compounds of the present
10 invention are effective as inhibitors of viral
polymerases at low micromolar concentrations.

It is apparent from the tables above that a compound of
the present invention which is effective in the
15 inhibition of one of the RNA dependent polymerases tested
may not necessarily be as effective in inhibiting the
other RNA dependent polymerases. The results shown in the
tables above indicate a general trend, although this is
not without exception. Generally, the most active
20 inhibitors of HCV RdRp contained a phenyl ring attached
to the diketoacid, whereas the HIV-RT inhibitors
contained a furanyl group and those of HBV polymerase a
thiophene group.

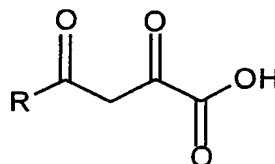
25

While not wishing to be bound by any particular theory,
the present inventors hypothesize that the diketoacid
fragment of the compounds of the present invention
inhibits RNA dependent polymerase activity by providing
30 an "active site anchor" and interacting with divalent

- 5 metal cations (Mg^{2+} , Mn^{2+}) required for polymerase activity. The ring system found on the left hand side of the molecule can apparently be modified in order to build specificity towards a given polymerase.

5 CLAIMS

1. The use of a compound of formula A, or of a
pharmaceutically acceptable salt or ester thereof,
wherein the group R is an organic moiety containing
10 2 to 24 carbon atoms which includes an optionally
substituted cyclic or heterocyclic group, and
wherein one of the atoms in the ring of the cyclic
or heterocyclic group is directly bonded to the
adjacent carbonyl in the diketoacid, in the
15 manufacture of a medicament for treatment or
prophylaxis of a viral illness in a human or animal
by inhibition of a viral polymerase.



20

FORMULA A

25

2. The use according to claim 1 wherein the medicament
is for the inhibition of a DNA polymerase or RNA
polymerase.

- 5 3. The use according to claim 1 or claim 2 wherein the medicament is for treatment or prevention of infection by an RNA virus, such as a positive single-stranded virus, negative single stranded virus or retrovirus, or a DNA virus.
- 10
4. The use according to claim 3 wherein the virus is selected from polio virus, hepatitis C virus, encephalomyocarditis, orthomyxoviruses, paramyxoviruses, HIV, and hepatitis B.
- 15
5. The use according to claim 3 wherein the medicament is for the inhibition of hepatitis C virus RNA dependent RNA polymerase (HCV RdRp), hepatitis B virus polymerase (HBV pol), or reverse transcriptase
- 20 of human immunodeficiency virus (HIV RT).
6. The use according to any one of the following claims wherein the group R is selected from:
- 25 (i) optionally substituted aromatic groups;
- (ii) optionally substituted heteroaryl groups;
- (iii) optionally substituted cycloalkyl groups;
- (iv) optionally substituted cycloalkenyl groups; and
- 30 (v) optionally substituted cyclic heteroalkyl

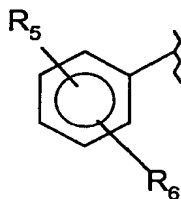
5 groups.

7. A compound of formula A, as set out in claim 1, or a
pharmaceutically acceptable salt or ester thereof,
for pharmaceutical use, wherein the group R is
10 selected from:

- (i) optionally substituted aromatic groups;
- (ii) optionally substituted heteroaryl groups;
- (iii) optionally substituted cycloalkyl groups;
- 15 (iv) optionally substituted cycloalkenyl
groups; and
- (v) optionally substituted cyclic heteroalkyl
groups, other than those containing a
single nitrogen in the ring.

20

8. A compound, ester or salt according to claim 7
wherein the group R is an optionally substituted
phenyl group of formula:



5

wherein R_5 and R_6 independently are selected from hydrogen and the following substituent groups:

- (a) -OH;
- (b) -SH;
- 10 (c) - halogen, such as fluorine, chlorine or bromine,
- (d) - CO_2H ;
- (e) - CN;
- (f) - NO_2 ;
- 15 (g) - NR_1R_2 wherein each of R_1 and R_2 is selected from H and lower alkyl groups having 1 to 6 carbon atoms; or R_1 and R_2 together form a ring including 4 to 6 carbon atoms;
- (h) - $\text{SO}_2\text{NR}_1\text{R}_2$ where R_1 and R_2 are as defined
20 above;
- (i) - CONR_1R_2 , - $\text{NR}_1\text{CO}_2\text{H}$, or - $\text{NR}_1\text{COCO}_2\text{H}$ where R_1 and R_2 are as defined above;
- (j) an alkyl (or alkenyl or alkynyl group) group
25 having 1 to 12 (2 to 12) carbon atoms,
preferably 1 to 7 (2 to 7) carbon atoms
optionally substituted by any one or more of
the groups (a) - (i) above and/or optionally
interrupted by a group selected from -O-, -S-,
- NR_3 -,

30

5

$\begin{array}{c} \text{O} \\ || \\ -\text{C}- \end{array}$, $-\text{CO}_2-$, $-\text{OCO}-$, $-\text{CONR}_3-$, $-\text{NR}_3\text{CONR}_3-$, $-\text{SO}_2$
 $-\text{NR}_3\text{SO}_2-$, and $-\text{SO}_2\text{NR}_3-$; where each R_3
independently is H or lower alkyl of 1 to 6 carbon
atoms;

10

(k) an aryl or heteroaryl group having 2 to 10
carbon atoms optionally substituted with any
one or more of groups (a) to (j) above;

15

(l) an aralkyl or heteroaralkyl group having 3 to
16 carbon atoms optionally substituted with any
one or more of groups (a) - (j) above and/or in
which the alkyl part of the group is optionally
interrupted by a group selected from

20

$-\text{O}-$, $-\text{S}-$, $-\text{NR}_3-$, $\begin{array}{c} \text{O} \\ || \\ -\text{C}- \end{array} - \text{CO}_2-$, $-\text{OCO}-$, $-\text{CONR}_3-$,
 $-\text{NR}_3\text{CONR}_3-$, $-\text{SO}_2-$, $-\text{NR}_3\text{SO}_2-$, and $-\text{SO}_2\text{NR}_3-$;
where R_3 is as defined above;

25

$\begin{array}{c} \text{O} \\ || \\ -\text{C}- \end{array} \text{R}_4$ where R_4 is an alkyl, alkenyl, alkynyl,
aryl, heteroaryl, aralkyl, or heteroaralkyl
group as such groups are defined above at (j),
(k) and (l);

30

5

(n) $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}-\text{R}_4 \end{array}$ or $\begin{array}{c} \text{O} \\ \parallel \\ -\text{O}-\text{C}-\text{R}_4 \end{array}$ where R_4 is as defined above;

(o) $-\text{OR}_4$ where R_4 is as defined above;

10

(p) $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{NHR}_4 \end{array}$, $\begin{array}{c} \text{O} \\ \parallel \\ -\text{NH}-\text{C}-\text{R}_4 \end{array}$ or $\begin{array}{c} \text{O} \\ \parallel \\ -\text{NH}-\text{C}-\text{NHR}_4 \end{array}$ where R_4 is as defined above;

(q) $-\text{SO}_2\text{R}_4$ where R_4 is as defined above;

15

(r) $-\text{NHR}_4$ or $-\text{N}(\text{R}_4)_2$ where R_4 is as defined above;

(s) $-\text{NHSO}_2\text{R}_4$ or $-\text{SO}_2\text{NHR}_4$, where R_4 is as defined above; and

20

(t) $-\text{SR}_4$

and each of optional substituents (j) to (t) above may optionally itself be substituted by one or more groups selected from (j) to (t).

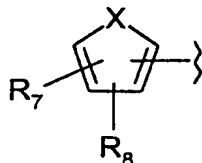
25

30

9. A compound, ester or salt according to claim 8 wherein the substituents R_5 and R_6 are independently selected from H-, -OH, $-\text{OR}_4$, $-\text{NHSO}_2\text{R}_4$, lower alkyl, aralkyl, amino, amide, urethane or urea groups.

- 5 10. A compound, salt or ester according to claim 8
 wherein the substituents R_5 and R_6 are independently
 selected from H-, -OH, -OR₄, and -NHSO₂R₄.
11. A compound, salt or ester according to claim 9 or
10 claim 10 containing only one substituent either of
 formula -OR₄ or -NHSO₂R₄.
12. A compound, salt or ester of any one of claims 9 to
 11 containing a group of formula -OR₄ and/or -NHSO₂R₄
15 selected from:
 -OCH₂Ar;
 -O(CH₂)₂Ar;
 -O(CH₂)₃CN;
 -O(CH₂)₃C≡CH; and
20 -NHSO₂Ar;
 wherein Ar is an optionally substituted aryl or
 heteroaryl group.
13. A compound, salt or ester, according to any one of
25 claims 8 to 12 having a single substituent at a
 position ortho- or meta- to the diketoacid group.
14. A compound, salt or ester according to any one of
 claims 8 to 12 having two substituents at the 2,5-;
30 3,5-; or 2,4-positions.

- 5 15. A compound, salt or ester according to claim 7
wherein the group of formula R has the formula:



and each of R₇ and R₈ is independently selected from hydrogen or from the list of substituent groups set
10 out at claim 8, and X is O, S, NH or NR₄, where R₄ is as defined above.

16. A compound, salt or ester according to claim 15
which is a pyrrole-2-substituted diketoacid, a
15 pyrrole-3-substituted diketoacid, a thiophene-2-substituted diketoacid, or a thiophene-3-substituted diketoacid.

17. A compound, salt or ester according to claim 16
20 which is a pyrrole substituted diketoacid in which each of R₇ and R₈ is hydrogen.

18. A compound, salt or ester according to any one of
claims 15 to 17 which is a pyrrole substituted
25 diketoacid having X=NR₄ and wherein R₄ is selected

- 5 from optionally substituted or interrupted, alkyl
 aryl or aralkyl groups.
19. A compound, salt or ester according to claim 7
 wherein R is selected from cyclopropyl-,
10 cyclopentyl-, cyclohexyl-, cyclopentenyl-,
 cyclohexenyl and adamantyl groups, any of which may,
 optionally, be substituted.
20. A pharmaceutical composition comprising a compound,
15 salt or ester of any one of claims 7 to 19 in
 combination with a pharmaceutically acceptable
 excipient, diluent or carrier.
21. Use, according to any one of claims 1 to 6, of a
20 compound, salt or ester according to any one of
 claims 7 to 19.
22. A use according to any one of claims 1 to 6 or 21
 wherein the medicament further comprises one or more
25 other agents for the treatment of viral infections.
23. A method of inhibiting a viral polymerase and/or of
 treating or preventing a viral illness by inhibiting
 a viral polymerase, the method comprising
30 administering to a human or animal subject suffering

- 5 from the condition a therapeutically or
prophylactically effective amount of the compound of
formula A set out in claim 1, or of a
pharmaceutically acceptable salt or ester thereof.
- 10 24. A compound of formula A, as set out in claim 1 or a
pharmaceutically acceptable salt or ester thereof
wherein the group R is selected from:
- (i) optionally substituted aromatic groups;
 - (ii) optionally substituted heteroaryl groups;
 - 15 (iii) optionally substituted cycloalkyl groups;
 - (iv) optionally substituted cycloalkenyl
groups; and
 - (v) optionally substituted cyclic heteroalkyl
groups, other than those containing a
20 single nitrogen in the ring.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB 99/02446

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C59/76 C07D207/30 C07D307/34 C07D333/04 A61K31/19
A61K31/335 A61K31/40 A61K31/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 475 109 A (SELNICK HAROLD G ET AL) 12 December 1995 (1995-12-12) cited in the application table 4	1-6, 24
X	----- TOMASSINI ET AL.: "Inhibition of" ANTIMICROB. AGENTS CHEMOTHERAP., vol. 38, no. 12, 1994, pages 2827-2837, XP002119719 table 1	1-14, 24
X	----- DE 32 14 082 A (ROUSSEL UCLAF) 4 November 1982 (1982-11-04) the whole document	7-14, 24
X	----- US 4 337 258 A (ROONEY CLARENCE S ET AL) 29 June 1982 (1982-06-29) claims 1-4	7, 24
	----- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

21 October 1999

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10/11/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Goetz, G

INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEILSTEIN INFORMATION SERVICE: FILE: XFIRE, XP002119720 see the compounds attached the whole document -----	24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/02446

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5475109 A	12-12-1995	GB 2294264 A,B US 5618830 A	24-04-1996 08-04-1997
DE 3214082 A	04-11-1982	FR 2504127 A AT 390054 B AT 144682 A AU 550822 B AU 8269082 A BE 892886 A CA 1168255 A CH 652387 A DK 170382 A,B, ES 511489 A FI 821305 A,B, FR 2526789 A GB 2096999 A,B GR 75457 A IE 52444 B IT 1147846 B JP 1020127 B JP 1536189 C JP 62174012 A JP 1429071 C JP 57183736 A JP 62037027 B LU 84090 A NL 8201579 A OA 7539 A PT 74767 A,B SE 453493 B SE 8201840 A SU 1264836 A US 4450292 A ZA 8202571 A	22-10-1982 12-03-1990 15-08-1989 10-04-1986 21-10-1982 18-10-1982 29-05-1984 15-11-1985 18-10-1982 01-02-1983 18-10-1982 18-11-1983 27-10-1982 19-07-1984 28-10-1987 26-11-1986 14-04-1989 21-12-1989 30-07-1987 09-03-1988 12-11-1982 10-08-1987 13-04-1983 16-11-1982 31-03-1985 01-05-1982 08-02-1988 18-10-1982 15-10-1986 22-05-1984 23-02-1983
US 4337258 A	29-06-1982	US 4423063 A	27-12-1983